EDITORIAL

Ion channels: their structure, function and control – an overview

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In the past two decades, investigations on ion channels have made remarkable progress. Since the development of patch clamp recording techniques in 1980s, investigators have been able to detect the real-time behaviour of single channels on many kinds of cell membrane. Rapid advances in molecular biology techniques led to the cloning and identification of a variety of ion channel genes, many of which have been the targets of knock-outs or disruptions. Recent significant progress in structural biology has enabled us to grasp the molecular architecture of some ion channels, and their structure–function relationship. In this special issue, knowledge from these various approaches are summarized and presented by seven specialists in the respective fields. This discussion took place on 24 March, 2003, during the joint annual scientific sessions of the 80th Japanese Physiological and the 76th Pharmacological Society meetings in Fukuoka, Japan. The symposium was held just after the outbreak of hostilities in Iraq, and some of the speakers originally invited could not participate. Fortunately, two excellent substitute speakers took part (Drs Zamponi and Findlay on Ca^{2+} channels).

Structure–function relationship of ion channels

In this year 2003, the Nobel Prize in Chemistry is dedicated to the discoveries concerning the structure–function relationship of ion and water channels in cell membranes. One laureate, Roderick MacKinnon, has been vigorously unravelling the molecular structures of several ion channels and providing structural insights into their biophysical properties. In this symposium, two experts in the field of structural biology discuss the structure–function relationship of ion and water channels. Eric Gouaux described the structure of the extracellular ligand-binding domain of ionotropic glutamate receptors (iGluR), and gave an account of the regulatory mechanisms of iGluRs, including desensitization, on a structural basis (Gouaux, 2003). Yoshinori Fujiyoshi has developed a powerful method for investigating 2D-crystal structure. In collaboration with Peter Agre, the second Nobel laureate, he has for the first time revealed the crystal structure of a water channel, aquaporin-1, and elegantly showed the permeation mechanism of water molecules. Although he provided an excellent lecture in the symposium, he was unable to contribute a review article to this series but aspects of this work are described in Agre *et al.* (2003). Some ion channels can be activated by intracellular ligands such as Ca^{2+}. Although John Adelman could not participate in the symposium, he has contributed an excellent review on his work on small conductance Ca^{2+}-activated K^+ (SK) channels to this series (Maylie *et al.* 2003). Unlike the classical Ca^{2+}-activated big conductance (BK) channel, SK channels are activated by binding of Ca^{2+} to calmodulin (CaM) covalently coupled with the channels. Using biochemical, molecular, electrophysiological and structural biological techniques, Adelman and colleagues have revealed the activation mechanism of SK channels by Ca^{2+}–CaM.

Controversies of Ca^{2+} channel inactivation

Voltage-dependent Ca^{2+} channels (VDCCs) are the major pathway of Ca^{2+} entry via excitable membranes. Upon depolarization, VDCCs open transiently, and this is followed by inactivation which shuts off voltage-dependent Ca^{2+} influx. Thus the inactivation properties of VDCCs are quite important for avoiding excess Ca^{2+} entry. In this issue, Gerald Zamponi and colleagues present a novel model for the molecular mechanism of voltage-dependent inactivation of VDCCs (Stotz *et al.* 2003). They suggest that the intracellular I–II linker region acts as a 'hinged-lid' and directly blocks the pore, in the same way as the 'ball-and-chain'

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model proposed earlier for voltage-dependent potassium channels. They also highlighted a possible involvement of the auxiliary β-subunit in voltage-dependent inactivation gating.

VDCCs undergo Ca^{2+}-dependent inactivation (CDI), as well as voltage-dependent inactivation (VDI). Which kind of inactivation mechanism is mainly used to shut off the channel under physiological conditions? Recently, Ian Findlay has answered this question (Findlay, 2003). In native cardiomyocytes, it had been thought that VDI was relatively slow and CDI played a major role. Findlay and colleagues found that depolarization increased the probability that an ion channel shows rapid VDI before CDI. He also shows that VDI is modulated by protein kinase A-mediated phosphorylation. The degree of VDI is attenuated and thus CDI plays a dominant role in the control of Ca^{2+} channel inactivation during β-adrenergic stimulation. The contribution of VDI and CDI to the decay of VDCCs is thus determined by turning on (by depolarization) or turning off (by adrenergic stimuli) of rapid VDI. These findings are intriguing for understanding physiological functions of VDCCs in action potential formation.

Diversity of inwardly rectifying potassium channels

Kir channels do not exhibit voltage-dependent gating mechanisms, but outward K^+ flow is blocked by intracellular non-organic or organic cations, such as Mg^{2+} or polyamines; this results in inward rectification. Kir channels are subject to various modulators, such as G-proteins or intracellular ATP.

The G-protein-gated inwardly rectifying K^+ (K_G, GIRK, Kir 3.1/3.4)) channel, which is directly activated by G-protein $\beta\gamma$ subunits released from pertussis toxin-sensitive G-proteins, is responsible for acetylcholine (ACh)-induced slowing of the heart, and for neurotransmitter-evoked slow inhibitory postsynaptic potentials in many different neurones. The native cardiac K_G channel current has been known to exhibit a characteristic voltage-dependent behaviour, called 'relaxation.' Yoshihisa Kurachi reported a series of studies showing that this behaviour reflects the apparent voltage-dependent control of the trimeric G-protein cycle by a family of regulators of G-protein signalling (RGS) proteins (Kurachi & Ishii, 2003). Their findings indicate that the control may not be restricted to K_G channels, but may also affect other G-protein effects such as adenylyl cyclase and phospholipase C. Thus it may be of general importance in the control of G-protein-mediated signalling systems.

The ATP-sensitive inwardly rectifying K^+ (K_{ATP}) channel, which is characterized by its inhibition by intracellular ATP, is distributed in various tissues, including pancreatic β-cells, heart, skeletal muscle, vascular smooth muscle and brain. This channel couples the cell metabolic state to membrane excitability. The K_{ATP} channel is a hetero-octamer composed of four pore-forming Kir6.x subunits (Kir6.1 or Kir6.2) and four regulatory sulphonylurea receptor subunits (SUR1, 2A and 2B). Susumu Seino has shown, by generating mice with one or other gene ablated, the physiological relevance of K_{ATP} channels containing Kir6.1 or Kir6.2 in various tissues (Seino & Miki, 2003). He reported that Kir6.2-containing K_{ATP} channels play a crucial role in the control of glucose metabolism, not only in pancreatic insulin secretion but also in glucose uptake by skeletal muscle, and in the control of glucagon secretion by the central nervous system. He and colleagues also showed that Kir6.2-containing K_{ATP} channels play crucial roles in protecting various organs including heart and brain again ischaemia. It is also noteworthy that their Kir6.1-knock-out mouse shows cardiac sudden death due to vasospasm, which confirms and establishes the importance of Kir6.1-containing K_{ATP} channels in the regulation of vascular tone, as proposed previously.

P2X receptor ion channels – their roles in nociception

ATP released from injured tissues is known to induce pain. The target of this ATP action is the P2X receptor, which constitutes a cation channel; activation by ATP causes a depolarization of peripheral afferent neurones. Alan North and colleagues have investigated the regulatory mechanisms of P2X receptor ion channels, since isolating the seven subunit cDNA clones (North, 2003). They are especially interested in channels consisting of heteromultimers of $P2X_2$ and $P2X_3$ subunits, for which studies in $P2X_3$-selective antagonists, antisense oligonucleotides and gene knock-outs support a role in neuropathic pain. By extensive use of the substituted cysteine accessibility method, they have deduced regions of the molecule that contribute to ion permeation and ligand binding. Experiments with intersubunit engineered disulphides indicate the arrangement of subunits in the heteromultimer channel.

Conclusions

This series of reviews on ion and water channels has concisely described current knowledge of their properties

from atomic structure to function. Ion and water channels are mandatory for both homeostasis and the dynamic activity of essentially all types of cells. Progress in basic research at various levels on these membrane proteins will facilitate our understanding of the beauty of life and will enable us to develop new ways to control cell functions to treat various diseases.

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